LCP, lamellar structures provide a more favorable setting in which GPCRs can oligomerize as a prelude to nucleation and crystal growth. These new findings lay the foundation for future studies of in meso crystallization mechanisms and for a rational approach to the generation of structure-quality crystals of membrane proteins.

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GPCRTooKit: A Computational Platform for Structural Comparison of GPCR Crystal Structures and Homology Model Refinement

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G-protein coupled receptors (GPCRs) constitute an important family of transmembrane receptors that regulate major life processes including sensory perception, cell proliferation and hormonal regulation. In lieu of the recent surge in GPCR crystal structures, structure based drug design methods are becoming more viable for GPCRs. However there is a huge disparity in the number of GPCR structures available and the number of GPCRs being studied in research. Thus homology models play an important role in a wide variety of GPCR research ranging from drug design to studying the functional mechanisms in these receptors. GPCR homology models do not capture the critical structural differences between the template and the GPCR being investigated.

We have developed GPCRToolKit that lays the computational framework for building GPCR based modeling tools. We have developed a robust and accurate homology model refinement method called LITiCon2.0, based on optimizing the helical translation, rotational orientation, tilt and gyration angles of the seven helices. The structural differences between two GPCRs stem mostly from the rigid body degrees of freedom. The algorithm is highly parallel to enhance the computational speed. We have tested and validated this method by refining the homology models of several class A GPCRs with known crystal structures, one of them being the chemokine CXCR4 using β2-adrenergic receptor as template. We have also analyzed the statistical distribution of translations, tilts and rotations of TM helices in all available GPCR crystal structures. We found that TM3 and TM7 showed relatively less variation in rigid body orientation compared to the other helices. Overall, TM4 and TM5 showed the highest diversity in spatial orientation among the TM domains. These results along with the description and validation of the LITiCon2.0 method will be presented.

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Rhodopsin Activation is Modulated by Non-Specific Membrane Lipid-Protein Interactions

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Rhodopsin activation proceeds through an ensemble of conformational substates: Meta I ↔ Meta II_a ↔ Meta II_b ↔ Meta II_bH^+ [1]. These substates are characterized by Schiff base deprotonation (Meta I), an outward tilt of helix H6 (Meta II_b), and protonation of Glu134 (Meta II_bH^+). Lipid bilayer composition has been shown to affect rhodopsin activation, providing an opportunity to systematically investigate membrane protein-lipid bilayer interactions on the mesoscale [2]. To quantify lipid acyl chain effects on GPCR activation, rhodopsin was reconstituted in lipids with symmetric unsaturated acyl chains (DOPC) or asymmetric lipids with *sn*-1 saturated and *sn*-2 unsaturated acyl groups (POPC). Proteolipid recombinant membranes were studied by UV-visible and FTIR spectroscopy. Symmetric membrane lipids (DOPC) stabilize Meta II_a and render the normally weakly populated substate accessible to study. The Meta II_a substate is characterized by an opening of the Schiff base ionic lock, and an activation switch in a conserved water-mediated, hydrogen-bonded network involving helices H1/H2/H7 that is sensed by Asp83. Replacement of an unsaturated acyl chain with a saturated chain (POPC) increases the pK_a value for the Meta I ↔ Meta II equilibrium, and consequently destabilizes the Meta II_a substate. Modulation of the bilayer curvature stress due to a negative monolayer spontaneous curvature (H_0) (DOPC) contributes a mechanical force that shifts the Meta I ↔ Meta II equilibrium towards Meta II, leading to rhodopsin activation. The flexible surface model (FSM) explains how chemically non-specific interactions between membrane proteins and the lipid bilayer contribute to GPCR activation [3,4].

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2384-Pos Board B154

Molecular Simulations Illuminate Rhodopsin Activation Based on New Crystal Structures

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The rhodopsin proteolipid system was modeled through all-atom molecular dynamics (MD) simulations on the microsecond timescale and compared to experimental ^{2}H NMR data. The post-isomerization behavior of the covalently bound ligand, retinal, was tested for two independent models: (i) the counterion-switch (neutral binding pocket) and (ii) complex-counterion (negative binding pocket) [1]. Each model leads to distinct geometrical rearrangements, with direct implications for interpretation of recent rhodopsin crystal structures [2,3]. The distinctive feature of the counterion-switch simulation entails fluctuations of the C11=C12-C13=C14 dihedral angle. These motions are correlated with changes in the C5-, C9-, and C13-methyl group orientations, and result in a long-axis flip of the polyene chain within the binding pocket of rhodopsin. In contrast, the complex-counterion simulation produced retinal fluctuations at the C7=C8-C9=C10 dihedral. This facilitates stabilization of the methyl group orientations ($\approx 60°$ with respect to the membrane normal), consistent with ^{2}H NMR results for the Meta I state [4]. Recently, a putative structure of the fully-activated Meta II state revealed a long-axis flip of the retinylidene chain relative to its orientation in the dark state [2]. Our simulations show that electrostatic changes to the rhodopsin binding pocket lead to alternate pathways of retinal conformational release. Agreement between simulation and spectroscopy indicates that the retinylidene flip may only occur in the Meta I ↔ Meta II transition. These simulations provide an essential framework for interpreting molecular snapshots (crystal structures) of membrane protein activation, illuminating how small-scale changes in a GPCR binding pocket can affect large-scale membrane protein-lipid bilayer dynamics.

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2385-Pos Board B155

Sampling of GPCR Second Extracellular Loops using Geometric Constraints

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Second extracellular loops (ECL2) of G protein-coupled receptors (GPCR) are known to play important roles by accommodating various GPCR ligands and providing ligand specificity. Despite the structural similarity among GPCR proteins, ECL2 structure is particularly hard to predict because of the relatively large size and ill-conserved sequence. In this study, we developed an efficient sampling algorithm for GPCR ECL2 that utilizes geometric constraints specific for GPCR. Two applications of the triaxial loop closure algorithm were employed to sample geometrically plausible ECL2 conformations that form a well-conserved disulfide bond with a particular transmembrane helix. Scores based on geometric constraints that effectively describe ECL2 environment were introduced to facilitate filtering of implausible ECL2 structures. All of these components are purely geometric, hence sampling and filtering can be performed with extremely low computational cost. A benchmark test was performed on seven unique GPCRs for which all-atom structures have been revealed. The result shows that the best model out of 50 sampled structures is of acceptable accuracy with the median loop RMSD less than 5 Å. Combined with energy-guided global optimization, further refined ECL2 structures could be obtained. New ideas introduced in this study may be useful for developing methodologies for further GPCR modeling and docking studies.

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Prediction of the Monomer-Monomer Interface Region of the ß2Ar Homodimer Receptor via Docking Experiments

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Increasing body of evidence indicate that G protein-coupled receptors (GPCRs) exist as dimers and oligomers in the cell membrane. The goal of this study is to estimate the homodimeric form of beta-2 adrenergic ($ß_2$AR) receptor which is a member of the GPCR family, via multiple docking experiments.

The transmembrane domain VI (TMVI) of the receptor is suggested to be a significant part of the interface both experimentally and theoretically [1,2]. The